1.114) according to a described method[14]. Isolated polymorphonuclear leukocytes (96-99% viability) were resuspended in HBSS with 15 mM phosphate buffer at room temperature and gently agitated on a shaker. The purity of this cell suspension was ≥ 85% polymorphonuclear leukocytes, ≤ 5% mononuclear cells and platelets, with the remaining percentage consisting of red blood cells. Purified polymorphonuclear leukocytes were used in the polarization assay. All incubation mixtures were maintained between an osmolality of 280 to 320, a pH range of 7.2 to 7.6, 15 mM phosphate buffer, and 50 μM Ca^{2+} and 50 μM Mg^{2+}.

EXAMPLE 7

Polarization Assay

Previous studies using the collagen gel-visual chemotactic assay[4] have shown N-acetyl-PGP to be a polymorphonuclear leukocyte chemoattractant. For chemotactic movement to occur the

cell must take on a polarized morphology, therefore, polarization is a necessity for chemotaxis. When polarization is prevented by an inhibitor, chemotaxis is necessarily inhibited. For this experiment, it was therefore chosen to rely on the polarization results directly.

The polarization assay[15] was performed in a blind fashion. This assay was used to determine the polymorphonuclear leukocyte response to chemoattractants and inhibitors by measuring the frequency and degree of cellular shape change. Briefly, 2×10^5 polymorphonuclear leukocytes were mixed with preincubated synthetic complementary peptides and chemoattractants in a reaction chamber (total volume = 100 µl) at 37°C for 5 min. At the end of the incubation period an aliquot was collected and mixed with an equal volume of 4% glutaraldehyde for microscopic observation. The remaining volume of each cell suspension was immediately centrifuged at 15,000 X g for 5 seconds to remove cells. The resulting supernatant was analyzed for lactic dehydrogenase activity.[16] All incubations generated lactate dehydrogenase activity correlating with <5 % cell death. Polymorphonuclear leukocytes in each sample were observed microscopically and assigned scores of 0

(resting = spherical cell with a smooth membrane), 1 (activated = irregular cell with uneven membranes) or 2 (polarized = cell length ≥ width X 2). Scores of 100 polymorphonuclear leukocytes for each sample were added to produce a polarization index. A dose response was performed for each chemoattractant. A concentration of each chemoattractant was chosen from the linear portion of each dose response curve and used as the positive control. Negative control samples consisted of polymorphonuclear leukocytes in HBSS only. Inhibition (ID_{50}) was expressed as the peptide concentration required to produce a 50% reduction in the polymorphonuclear leukocyte polarization response to the chemoattractant. The Student's *t*-test (unpaired) was used to analyze the differences in the mean polarization response between polymorphonuclear leukocytes activated with the chemoattractant in the absence or presence of complementary peptides.